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Cloning and Structure of Different Types of Spider Silk

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Based on the amino acid sequence of peptides derived from Nephila dragline silk we used DNA probes to identify several clones from a silk gland cDNA library. The largest of these clones (2.5kb) has been partially (80%) sequenced. The sequence contains the poly A site, 340 base of 3' untranslated region and the rest protein coding region. The protein region consists of a basic 34 amino acid repeat. The repeat itself consists of three regions. The first consists of 0-9 amino acids with a sequence of $AGR(GGX)_{\frac{1}{2}}^{\uparrow}$. Clearly this region is not highly conserved. The second region has a sequence of $GAG(A)_{\frac{1}{x}}^{\uparrow}$ which is highly conserved in all repeats. The third segment is $(GGX)_{5}^{\uparrow}$ and is very highly conserved. Clones for other silk proteins are in various stages of isolation and sequencing.

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PROGRESS REPORT on N00014-89-J-1564 for December 1, 1989

PRINCIPAL INVESTIGATOR: Randolph V. Lewis
CONTRACTOR: University of Wyoming
CONTRACT TITLE: Cloning and Structure of Spider Silk
START DATE: December 1, 1988

RESEARCH OBJECTIVES: Clone, sequence and express dragline silk protein from Nephila Clavipes and compare the sequence to clones of the same protein from Areneus gemmoides.

PROGRESS: We have sequenced over 2 kb of two separate clones for the Nephila dragline silk protein. One of the clones has the poly A tail and thus we have the 3' end of the mRNA in that clone. From this data we have compiled a tentative repeating protein segment. At this point it is clear that the repeats are not exact although common features are beginning to emerge. There appear to be three regions to the repeat. The first is highly variable in both length and sequence ranging from 0 to 9 amino acids. The sequence is $AGR(GGX)_2$. The second consists of $GAG(A)_X$ and is 8 to 10 amino acids long. This segment is well conserved in all repeats. The third is highly conserved consisting mainly of a G-G-X and is 15 amino acids in length. In most cases X is A,Q Y or L. The sequence determined to date contains all but one of the peptides we have sequenced from the silk fibers.

We have started inserting the longest clone into an expression vector and sequenced the vector to insure that the insert is in frame. So far none of the clones have contained the insert in frame even though the procedure is designed to put it in predominantly in frame. We will begin expression studies looking for the silk fusion protein as soon as we have the in frame insert.

We have screened a cDNA library for the dragline silk protein from Areneus and have several positive clones which also were positive in a southern blot of the inserts. Since all the inserts were less than 500 bp we are making another library with size selection for those inserts larger than 1000bp only. In addition through protein sequencing of fragments derived from an N-bromo succinimide cleavage we have determined that the protein sequence of, at least, some part of the protein is very similar to that seen in Nephila. We have also generated a cDNA library from the spider abdomen to have a library for the other silk

type proteins. We are currently using the polymerase chain reaction to amplify the specific cDNA prior to cloning. The procedure seems to be working very well as only 4 clones have amplified from the whole abdomen cDNA for both the cocoon and minor ampullate silk probes. These will each be sequenced to insure that the proper cDNA is being studied.

We have synthesized peptides corresponding to the complete repeat segment and to the third, highly conserved, region. These are being used in biophysical studies primarily with CD and FTIR.

WORK PLAN(year 2): We will complete the sequence of Nephila dragline silk. Due to problems in the nested deletion method we are going to use partial restriction digests to obtain the segments of missing sequence. The work to express this protein will continue with the expected result being a plasmid we know is correct and beginning to assess conditions for expression. We should have enough sequencing completed soon to insure that we have the proper Areneus major ampullate silk, Nephila cocoon and minor ampullate silk clones and begin to identify the repeated segments of that silk. We will also pursue the use of the peptides we have synthesized to understand the structure of the protein and how it forms the fiber structure.

INVENTIONS: We submitted our information to Research Corp. for their analysis of the patent possibilities. They initially declined to pursue it until more sequence was known and evidence of expression is shown. We recently met with them and they have decided to begin work on a patent application with the goal of having everything ready by the time we complete our cDNA sequence.

PUBLICATIONS:

1. Dong, Xu, Lewis, and Middaugh(1989) The Molecular Basis of Spider Silk Elasticity. submitted to JMB and under review there.

TRAINING ACTIVITIES: Two graduate students are working on this project as well as one undergraduate. In addition, a research assistant professor joined the group last month.

Women and minorities 1

Non-citizens 2 (both from China)

AWARDS: None.

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